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(54) **ANTIBIOTIC PRODUCING MICROBE**

ANTIBIOTIKA PRODUZIERENDE MIKROBE

MICROBE PRODUISANT UN ANTIBIOTIQUE

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(56) References cited:
- **ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 34, no. 1, January 1990, pages 121-127, XP002040085 CHARLES E. ISAAC ET AL.: "Production of cyclosporins by Topocladium niveum strains" cited in the application**

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Description

Field of the Invention

[0001] The present invention relates to a novel cyclosporin-producing microbe, apparently a member of the fungal genus *Tolypocladium*, and to a process for producing cyclosporins by the fermentation of this microbe.

Background of the Invention

[0002] Cyclosporins are a group of non-polar cyclic oligopeptide compounds having immunosuppressant activity. Cyclosporins are produced by fungal fermentation. Cyclosporins have been employed for several years to combat rejection of transplanted organs and tissues in humans. Recently, investigators have been seeking additional therapeutic applications for the drug.

[0003] At least nine different cyclosporins are produced by the fungi, including cyclosporin A, B, C, D, E, F, G, H, and I, all having varying amino acid composition. Cyclosporin A is the major component and currently enjoys the most wide-spread clinical use. *See, e.g.,* Ruegger et al., *Helv. Chim. Acta* 59:1075 (1976); Traber et al., *Helv. Chim. Acta* 60:1568 (1977); Rehacek and De-xiu, *Process Biochem* 26:157 (1991). Cyclosporin A is commercially available under the tradename SANDIMMUNE® from Sandoz Corp.

[0004] Cyclosporins were originally isolated as metabolites produced by fungal strains of *Cylindrocarpon lucidum* Booth and *Tolypocladium inflatum* Gams (Gams, *Persoonia* 6:185 (1971)), isolated from soil samples from the United States of America and Norway. *T. inflatum* is also known as *Tolypocladium niveum.* Isaac et al., *Antimicro. Agents Chemother*. 34:121 (1990). (The genus *Tolypocladium* was first described by Gams, *Persoonia,* 6:185 (1971); the genus name *Beauveria* has been used synonymously with *Tolypocladium*.) Species of Tolypocladium are typically slow in growth and form white, flocculent colonies with a large amount of spores.

[0005] A production strain of *Tolypocladium* (*Tolypocladium inflatum* (NRRL 8044)) was at first identified as *Trichoderma polysporum* (Link ex Pers) Rifai. The use of this strain for the production of antibiotic substances is described in Finnish Patent No. 54606. Growth conditions and taxonomy of this production strain are provided in Dreyfuss et al., *Eur. J. Appl. Microbiol.* 3:125 (1976).

[0006] Other cyclosporin producing strains are described in Finnish Patent No. 52851 (*Cylindrocarpon lucidum* Booth (NRRL 5760)), German patent 298276 (*Tolypocladium inflatum* strain SF 136), Great Britain patent 2,227,489 (*Tolypocladium varium*), Japan patent 826 3093 (two strains of cyclosporin producing *Fusarium*), and US patent No. 5,409,816 (*Tolypocladium* sp. LEA3). However, not all strains of *Tolypocladium* species are cyclosporin producing. Isaac et al. (1990).

[0007] Cyclosporin production varies among species and strains, and production processes for cyclosporin A have encountered problems with low yields or the need for long fermentation times. Even where a microbial strain has been shown to produce relatively high yields of cyclosporin, the relative amount of cyclosporin produced often is small. Dexiu et al., *Folia Microbiol*. 36:549-556 (1991). The cyclosporin production of some known strains of Tolypocladium are compared in Isaac et al., *Antimicro. Agents Chemother.* 34:121 (1990). Of the nine cyclosporin producing strains compared in Isaacs et al., total cyclosporin production (forms A, B and C) after 10-15 days of growth ranged from 12 mg/liter to 123 mg/liter, using their described production process. A mutant of *T*. *inflatum* NRRL 8044 is described as producing about 500 mg of Cyclosporin A per liter in Traber et al., *J. Antibiotics* 42:591 (1989). Slight improvements in yield have been reported using selected amino acids or carbon sources, however, the operating expense of such methods renders them unsuitable for large-scale commercial use. *See, e.g*., Agathos et al., Ann. NY *Acad. Sci.* 506: 657 (1987); Kobel et al. *Europ. J. Appl*. *Microbiol*. *Biotechnol*. 14:237 (1982). Accordingly, it is desirable to identify new strains of cyclosporin-producing microbes which can be used efficiently in the production of cyclosporins.

Summary of the Invention

[0008] It is an object of the present invention to provide a productive cyclosporin-producing microbe.

[0009] It is a further object of the present invention to provide a biologically pure culture of a productive cyclosporin-producing microbe having the identifying characteristics of the deposit having provisional accession number I-1714, made in accordance with the provisions of the Budapest Treaty on 28 May 1996, with the Collection Nationale de Cultures de Microorganisms, Institut Pasteur, 25 Rue du Docteur Roux, Paris.

[0010] Another object of the present invention is a process for producing cyclosporin, wherein a microbe strain having all of the identifying characteristics of the deposit having provisional accession number I-1714 (Collection Nationale de Cultures de Microorganisms, Institut Pasteur, Paris) and referred to herein as *Tolypocladium* sp. (Poli), is aerobically fermented in a nutrient medium containing a carbon source utilizable by the microbe strain until cyclosporin is produced, and the cyclosporin produced is recovered.

[0011] The foregoing and other objects and aspects of the present invention are explained in detail in the specification set forth below.

Brief Description of the Drawings

[0012]

FIGURE 1A is a photograph of a colony of one strain of *Tolypocladium* sp. (Poli).
FIGURE 1B is a photograph showing the morphology of a strain of *Tolypocladium* sp. (Poli).
FIGURE 2A is a photograph of a colony of *Tolypocladium inflatum* ATCC 34921 (NRRL 8044).
FIGURE 2B is a photograph showing the microscopic morphology of *Tolypocladium inflatum* ATCC 34921 (NRRL 8044).
FIGURE 3A is a chromatograph showing the production of cyclosporin by fermentation of *Tolypocladium* sp (Poli).
FIGURE 3B is a chart tabulating the data represented in the chromatograph of Figure 3A.
FIGURE 4A is a chromatograph showing the production of cyclosporin by fermentation of *Tolypocladium* sp NRRL 8044 (ATCC 34921).
FIGURE 4B is a chart tabulating the data represented in the chromatograph of Figure 4A.

Detailed Description of the Invention

[0013] The novel microbe described herein provides a high yield of cyclosporin within a relatively short fermentation time, and is referred to herein as *Tolypocladium* sp. (Poli). This novel microbe was isolated from a soil sample obtained in Russia. The original isolate initially obtained from the soil sample possessed the general characteristics of the fungal genus Tolypocladium (as described in Gams, *Persoonia* 6:185 (1971) and Bissett, *Can. J. Bot.* 61:1311 (1983)): short cylindrical conidiophores with terminal and lateral phialides, ellipsoidal to subglobose basally and narrowing into a cylindrical neck, often crooked, and bearing subglobose conidia. This original strain produced a low yield of cyclosporin antibiotic complex. After repeated mutagenesis and selection a new microbial strain was isolated with a distinct macroscopic appearance of the colonies and distinct biochemical characteristics. This strain is termed *Tolypocladium* sp. (Poli), and pure cultures have been grown. Specimens of *Tolypocladium* sp. (Poli) have been deposited on 28 May 1996 according to the Budapest Treaty at the Collection Nationale de Cultures de Microorganismes, Institute Pasteur, 25 Rue du Docteur Roux, Paris, France (accession number I-1714).

[0014] The novel microbe described herein is referred to as *Tolypocladium* sp. (Poli) and, while not wishing to be limited to a single theory of taxonomy, the data suggest that this microbe is a novel species of *Tolypocladium*. The novel microbe of the present invention possesses all of the general characteristics of the genus *Tolypocladium* yet is distinct from *Tolypocladium inflatum* (Gams) in morphology and in cyclosporin production. Alternatively, the novel microbe of the present invention may be termed a distinct strain of *Tolypocladium inflatum*.

[0015] *Tolypocladium* sp. Poli was compared to various species and strains of cyclosporin-producing microbes to establish its distinct characteristics. *Tolypocladium* sp. (Poli) is slow-growing, forming cream colored colonies from 3-8 mm in diameter, with a wrinkled appearance when grown for 15 days at 24°C on a solid medium containing Beet molasses (1.5%), glycerol (1.5%), peptone (0.6%), sodium chloride (0.5%) and other mineral salts and trace elements. The vegetative hyphae are composed of chains of swollen cells, and spore-forming ability is weak. Conidiophores are hyaline, cylindrical, usually arising as short, irregular branches from the aerial mycelium. Phialides are solitary or in verticils, ellipsoidal to subglobose basally, and narrowing abruptly into a cylindrical neck of variable length. The phialides become elongated toward the end of their development.

[0016] *Tolypocladium* sp. (Poli) can efficiently utilize the following as carbon sources: cellobiose, maltose, trehalose, galactose, glycerol, melezitose, sucrose, sorbitol, D-xylose, dextrin, and fructose. The following can also be utilized as carbon sources by *Tolypocladium* sp. (Poli), although less efficiently: mannitol and glucosamine. None of the following are utilized by *Tolypocladium* sp. (Poli) as a carbon source: L-arabinose, inositol, lactose, raffinose, xylitol, lactulose, stachyose, D-tagatose.

[0017] *Tolypocladium* sp. (Poli) can utilize either organic or inorganic nitrogen sources, including but not limited to corn steep liquor; dry yeast; protein hydrolysates (peptones) derived from casein, liver, lactalbumen, yeast, blood, meat and soya; vegetable meals (soya bean, cotton seed, corn gluten); urea and ammonium salts (nitrates, phosphates, sulphates).

[0018] *Tolypocladium* sp. (Poli) may be cultivated on various nutrient media containing the typical nutrients for fungi, including a carbon source suitable for (i.e., utilizable by) the microbe, a nitrogen source, and mineral salts and trace elements as are known in the art. As used herein, a medium capable of supporting *Tolypocladium* sp. (Poli) is one which contains a carbon source utilizable by the microbe and a nitrogen source utilizable by the microbe. Such a medium may be natural, synthetic or semisynthetic. Numerous compositions useful as carbon sources and nitrogen

sources in culture media are well known in the art. In addition, inorganic salts may be added to the culture medium as necessary, as is known in the art.

[0019] In a process according to the present invention for the production of cyclosporins, and particularly cyclosporin A, *Tolypocladium* sp. (Poli) is first cultured aerobically for 48-72 hours in a growth medium containing carbon and nitrogen sources utilizable by *Tolypocladium* sp. (Poli) at temperatures from about 20°C to about 30°C, preferably at about 24-25°C. The growth medium is inoculated with a suspension of conidia and/or mycelia from *Tolypocladium* sp. (Poli). A fraction of this initial culture, such as 10-20%, is then used to inoculate a production medium containing carbon and nitrogen sources utilizable by the microbe. Precursor amino acids may be added to the medium in order to improve the production of cyclosporin or to vary the relative amounts of the different cyclosporins, as is known in the art and as would be apparent to one skilled in the art. Aerobic fermentation is carried out at temperatures of from about 20°C to about 25°C (preferably at about 24 °C) for times of from about 8 to about 11 days. Commercially available fermentation devices may be used in the present process, as would be apparent to one of ordinary skill in the art. Cyclosporin production may be monitored using methods known in the art including, for example, measurement of antifungal activity of the culture broth or chromatographic analysis. Fermentation is stopped when a maximum or desired cyclosporin production is obtained. The desired cyclosporins are then recovered from the culture broth using methods known in the art and purified as needed. *See, e.g.,* US Patent No. 4,215,199; US Patent No. 5,156,960.

[0020] Use of the novel microbe of the present invention, here termed *Tolypocladium* sp. (Poli), according to the present invention provides a high yield of cyclosporin antibiotic complex. Yields of up to 1.5 g/L, 2.0 g/L, and even 3.0 g/L and above may be obtained. The cyclosporin produced by *Tolypocladium* sp. (Poli) contains cyclosporin A as the main product (more than 50%), and contains cyclosporins B, C and D as minor components. Up to 60%, 70% and even 80% or more of cyclosporin A may be obtained. The relative amounts of the different cyclosporins may be varied somewhat by maintaining the pH of the culture at different values between pH 3 - pH 7, such as by adding a diluted ammonia solution (as would be apparent to one skilled in the art).

[0021] An advantageous cyclosporin producing process is described in the co-pending United States patent application of B. Bocchiola, V. Buran and A. Magni, filed concurrently herewith.

[0022] The following examples are provided to illustrate the present invention, and should not be construed as limiting thereof. In these examples, g means gram, L means liter, mL means milliLiter, rpm means revolutions per minute, VVM means volume of air per unit volume of fermentation broth per minute, °C means degrees centigrade, and mcg means microgram.

EXAMPLE 1

Carbon Sources

[0023] The ability of *Tolypocladium inflatum* ATCC 34921 (NRRL 8044) and *Tolypocladium* sp. (Poli) to utilize varied carbon sources was directly compared by growing cultures under identical conditions. The ability of *Tolypocladium* sp. LEA3 (U.S. Patent No. 5,409,816) to utilize various carbon sources was obtained from published sources.

[0024] *Tolypocladium inflatum* ATCC 34921 (NRRL 8044) and *Tolypocladium* sp. (Poli) strains were compared in side-by-side growth tests using a liquid semi-synthetic medium containing corn steep powder (1 g/L), urea (1 g/L) and mineral salts (2 g/L KH_2PO_4; 3 g/L NaCl; 0.5 g/L $MgSO_4$; 1 g/L $NaNO_3$; 0.5 g/L KCl; 0.015 g/L $FeSO_4$; 0.003 g/L $MnSO_4$; 0.003 $ZnSO_4$; 0.001 $CuSO_4$), with different carbon sources added to a final concentration of 30 g/L. Shake flasks containing 25 mL of medium were inoculated with a suspension of conidia and mycelia and incubated for 4 days at 24°C on a gyratory shaker at 240 rpm. Growth was assessed by measuring the dry weight in each flask after 4 days, compared with a control flask containing the same medium without a carbon source, inoculated with the same suspension.

[0025] Information provided in **Table** 1 regarding *Tolypocladium* sp. LEA3 was obtained from U.S. Patent No. 5,409,816.

[0026] As shown in **Table** 1, *Tolypocladium* sp. (Poli) was unable to use L-arabinose, inositol, and xylitol, whereas *Tolypocladium* sp. LEA3 was able to utilize each of these compounds as a carbon source. In contrast to *T. inflatum* ATCC 34921 (NRRL 8044), T. (Poli) was unable to use inositol or xylitol as a carbon source.

TABLE 1

Carbon Source	*Tolypocladium* sp. (LEA3)	*Tolypocladium* sp. (Poli)	*T. inflatum* ATCC 34921 (NRRL 8044)
Control		-	-
L-arabinose	+	-	-
Cellobiose	+	+	+

TABLE 1 (continued)

Carbon Source	*Tolypocladium* sp. (LEA3)	*Tolypocladium* sp. (Poli)	*T. inflatum* ATCC 34921 (NRRL 8044)
Inositol	+	-	+/-
Lactose	-	-	-
Maltose	+	+	+
Raffinose	-	-	-
Trehalose	+	+	+
Xylitol	+	-	+
Galactose	+	+	+
Glycerol	+	+	+
Melezitose	+	+	+
Sucrose	+	+	+
Sorbitol	+	+	+
D-Xylose	+	+	+
Dextrin	(nd)	+	+
Fructose	(nd)	+	+
Mannitol	(nd)	+/-	+
Glucosamine	(nd)	+/-	+/-
Lactulose	(nd)	-	-
Stachyose	(nd)	-	(nd)
D-tagatose	(nd)	-	(nd)

(-) indicates a dry weight of < 1 g/L
(+/-) indicates a dry weight of between 1 and 2 g/L
(+) indicates a dry weight of > 2 g/L
(nd) indicates this test was not done

EXAMPLE 2

Comparison of Colony Growth and Fungal Morphology

[0027] The growth and characteristics of colonies of *Tolypocladium* sp. (LEA3), *Tolypocladium inflatum* ATCC 34921 (NRRL 8044), and *Tolypocladium* sp. (Poli) were compared, as was fungal morphology.

[0028] Fungal morphology of *Tolypocladium inflatum* ATCC 34921 (NRRL 8044) and *Tolypocladium* sp. (Poli) were compared after growth under identical conditions: microbes were grown directly on a sterile microscope slide, partially submerged in a liquid medium containing glucose (1.5%), glycerol (3%), yeast extract (0.5%), KH_2PO_4 (0.5%) and NaCl (0.5%) at room temperature. After 5 and 10 days the aerial mycelium were observed using a microscope. Description of *Tolypocladium* sp. (LEA3) was obtained from the published literature. Results are provided in **Table 3.**

[0029] **Table 2** compares additional characteristics of the three microbe strains. *Tolypocladium* sp. (Poli) can be distinguished from each of the other two strains as shown, for example, by comparing colony appearance, the forms of cyclosporin produced, and morphology. Growth was assessed subjectively using visual inspection and measurement. Colonies of *Tolypocladium inflatum* ATCC 34921 (NRRL 8044), when grown on a solid medium, are visible after 4-5 days while colonies of *Tolypocladium* sp. (Poli) are visible after one week. When these two microbial strains are grown in the same conditions using a liquid medium containing a suitable carbon source and identical inoculum, the growth curve obtained by plotting the dry weight values at different times are different, with *Tolypocladium inflatum* ATCC 34921 (NRRL 8044) growing faster than *Tolypocladium* sp. (Poli).

TABLE 2

	Growth	Cyclosporins	Spore-forming Ability	Colony Appearance	Odor	Hyphae Morphology
T. inflatum NRRL 8044	rapid	A B C D	Strong	white, floccose, unwrinkled	indistinct	hyaline, thin, elongated and branched
Tolypocladium (Poli)	slow	A B C D	Weak	cream, wrinkled	indistinct	swollen cells, chains
T. sp LeA3	rapid	A B C D G	Weak	greyish	indistinct	swollen cells, chains

Growth of Poli and NRRL 8044 was assessed by visual inspection and measurement; "rapid" and "slow" are comparative assessments.

Spore-forming ability of Poli and NRRL 8044 was assessed visually using a microscope.

Colony appearance of Poli and NRRL 8044 was assessed by visual, side-by-side comparison.

TABLE 3

	Conidiophore Morphology	Phialides Morphology	Conidia Morphology
T. inflatum NRRL 8044 (ATCC 34921)	hyaline, cylindrical, usually arising as short branches from the aerial mycelium, irregularly branched	solitary or in verticils, ellipsoidal to subglobose basally, narrowing abruptly into a cylindrical neck with varied length, frequently bowed; become elongated at the end of their development	description not possible
Tolypocladium (Poli)	hyaline, cylindrical, usually arising as short branches from the aerial mycelium, irregularly branched	solitary or in verticils, ellipsoidal to subglobose basally, narrowing abruptly into a cylindrical neck with varied length, frequently bowed; become elongated at the end of their development	description not possible
T. sp LeA3	not described	not described	irregular

[0030] A colony of one strain of *Tolypocladium* sp. (Poli) is shown in **Figure 1A**; a colony of *Tolypocladium inflatum* ATCC 34921 (NRRL 8044) is shown in **Figure 2A** for comparison. **Figure 1B** is a photograph showing the morphology of a strain of *Tolypocladium* sp. (Poli); **Figure 2b** is a photograph showing the microscopic morphology of *Tolypocladium inflatum* ATCC 34921 (NRRL 8044).

EXAMPLE 3

Cyclosporin Production by *Tolypocladium* sp. (Poli)

[0031] *Tolypocladium* sp (Poli), as described above, was cultured and the production of cyclosporin was assessed. The microbe was cultured using a seed medium containing sucrose and a production medium containing glucose was utilized. Cyclosporin A was separated from other forms of cyclosporin and other fermentation byproducts using chromatography. Using identical methods, *Tolypocladium* sp NRRL 8044 (ATCC 34921) was cultured and the production of cyclosporin was assessed. Growth and fermentation were carried out as follows: A shake flask of 2000 mL capacity was filled with 500 mL of a seed medium containing 2% sucrose, 1.5% glycerol, 0.7% ammonium sulfate, 0.5% sodium chloride and 0.2% potassium phosphate. Flasks were inoculated with a suspension of mycelium and conidia obtained from a piece of a 14-20 day old agar slant culture, chopped in 6-10 mL of water. Flasks were incubated for 2-3 days at 20°C to 30°C (preferably at 24°C) on an alternative shaker. Flasks were then used to inoculate a fermentor of 10 L capacity, filled with 5 L of the same seed medium. The seed culture was cultivated for 2-3 days at 20°C to 30°C (preferably at 24°C), and used as inoculum for the production medium (composed of glucose, glycerol, peptone, urea, sodium nitrate, ammonium sulfate, potassium phosphate, and other mineral salts and trace elements as are known in the art). In a convenient method, a 10% to 20% v/v of the seed culture was transferred in the production medium. Fermentation was performed at 20°C to 25°C (preferably at 24°C) for 10-14 days. The aerobic conditions were maintained by stirring (60 to 120 rpm) and aerating the broth at 0.5 to 1 VVM (volume of air per unit volume of fermentation broth per minute).

[0032] The cyclosporin concentration of broth was 7measured by High Performance Liquid Chromatography (HPLC) and the fermentation was stopped when cyclosporin production reached a maximum. The results of HPLC for *Tolypocladium* sp (Poli) are provided in **Figures 3A** and **3B**; that for *Tolypocladium* sp NRRL 8044 (ATCC 34921) is provided in **Figures 4A** and **4B**.

[0033] As shown by the HPLC results, the cyclosporin A overall yield using *Tolypocladium* sp (Poli) was more than 2 g/liter. The cyclosporin A overall yield using *Tolypocladium* sp NRRL 8044 (ATCC 34921) was less than 0.5 g/liter. In published reports, fermentation of *Tolypocladium* sp. LEA3 is stated as producing up to 1.5 g/l of cyclosporin A after six days of fermentation, with the relative proportion of cyclosporin A up to 84% (US Patent No. 5,409,816).

[0034] The foregoing is illustrative of the present invention and is not to be construed as limiting thereof. The invention is defined by the following claims.

Claims

1. A biologically pure culture of the cyclosporin-producing microbe deposited as provisional accession number I-1714, Collection Nationale de Cultures de Microorganisms, Institut Pasteur.

2. A process for producing cyclosporin, comprising aerobically fermenting a microbe having all of the identifying characteristics of the microbe deposited as provisional accession number I-1714, Collection Nationale de Cultures de Microorganisms, Institut Pasteur, in a nutrient medium comprising a carbon source utilizable by said microbe, until cyclosporin is produced and recovering the cyclosporin produced.

3. The process according to claim 2 wherein the cyclosporin recovered is cyclosporin A.

4. The process according to claim 2 wherein said nutrient medium comprises a carbon source selected from the group consisting of cellobiose, maltose, trehalose, galactose, glycerol, melezitose, sucrose, sorbitol, D-xylose, dextrin, fructose, mannitol and glucosamine.

5. The process according to claim 2 wherein said nutrient medium comprises glucose, glycerol, peptone, and ammonium sulfate.

6. The process according to claim 2 wherein the nutrient medium contains urea in a concentration of from about 0.01% to about 3.0% weight/weight.

Patentansprüche

1. Biologisch reine Kultur der Cyclosporin-erzeugenden Mikrobe, die als vorläufige Hinterlegungsnummer I-1714 bei

der Collection Nationale de Cultures de Microorganisms, Institut Pasteur, hinterlegt ist.

2. Verfahren zur Erzeugung von Cyclosporin, umfassend aerobisches Fermentieren einer Mikrobe, die sämtliche der identifizierenden Eigenschaften der Mikrobe aufweist, die als vorläufige Hinterlegungsnummer I-1714 bei der Collection Nationale de Culture de Microorganisms, Institut Pasteur, hinterlegt ist, in einem Nährmedium, das eine Kohlenstoffquelle umfasst, die von der Mikrobe nutzbar ist, bis Cyclosporin erzeugt wird und Gewinnen des erzeugten Cyclosporins.

3. Verfahren nach Anspruch 2, wobei das gewonnene Cyclosporin Cyclosporin A ist.

4. Verfahren nach Anspruch 2, wobei das Nährmedium eine Kohlenstoffquelle umfasst, die ausgewählt ist aus der Gruppe bestehend aus Cellobiose, Maltose, Trehalose, Galactose, Glycerin, Melezitose, Sucrose, Sorbit, D-Xylose, Dextrin, Fructose, Mannit und Glucosamin.

5. Verfahren nach Anspruch 2, wobei das Nährmedium Glucose, Glycerin, Pepton und Ammoniumsulfat umfasst.

6. Verfahren nach Anspruch 2, wobei das Nährmedium Harnstoff in einer Konzentration von etwa 0,01% bis etwa 3,0% Gew./Gew. enthält.

Revendications

1. Culture pure du point de vue biologique du microbe produisant de la cyclosporine déposé sous le numéro d'accès provisoire I-1714, Collection Nationale de Cultures de Microorganismes, Institut Pasteur.

2. Procédé pour la production de cyclosporine, comprenant la fermentation aérobie d'un microbe ayant toutes les caractéristiques d'identification du microbe produisant de la cyclosporine déposé sous le numéro d'accès provisoire I-1714, Collection Nationale de Cultures de Microorganismes, Institut Pasteur, dans un milieu nutritif comprenant une source de carbone utilisable par ledit microbe, jusqu'à la production de cyclosporine, et la récupération de la cyclosporine produite.

3. Procédé selon la revendication 2, dans lequel la cyclosporine récupérée est la cyclosporine A.

4. Procédé selon la revendication 2, dans lequel ledit milieu nutritif comprend une source de carbone choisi dans le groupe consistant en cellobiose, maltose, tréhalose, galactose, glycérol, mélézitose, sucrose, sorbitol, D-xylose, dextrine, fructose, mannitol et glucosamine.

5. Procédé selon la revendication 2, dans lequel ledit milieu nutritif comprend du glucose, du glycérol, une peptone et du sulfate d'ammonium.

6. Procédé selon la revendication 2, dans lequel ledit milieu nutritif contient de l'urée à une concentration d'environ 0,01% à environ 3,0% en poids/poids.

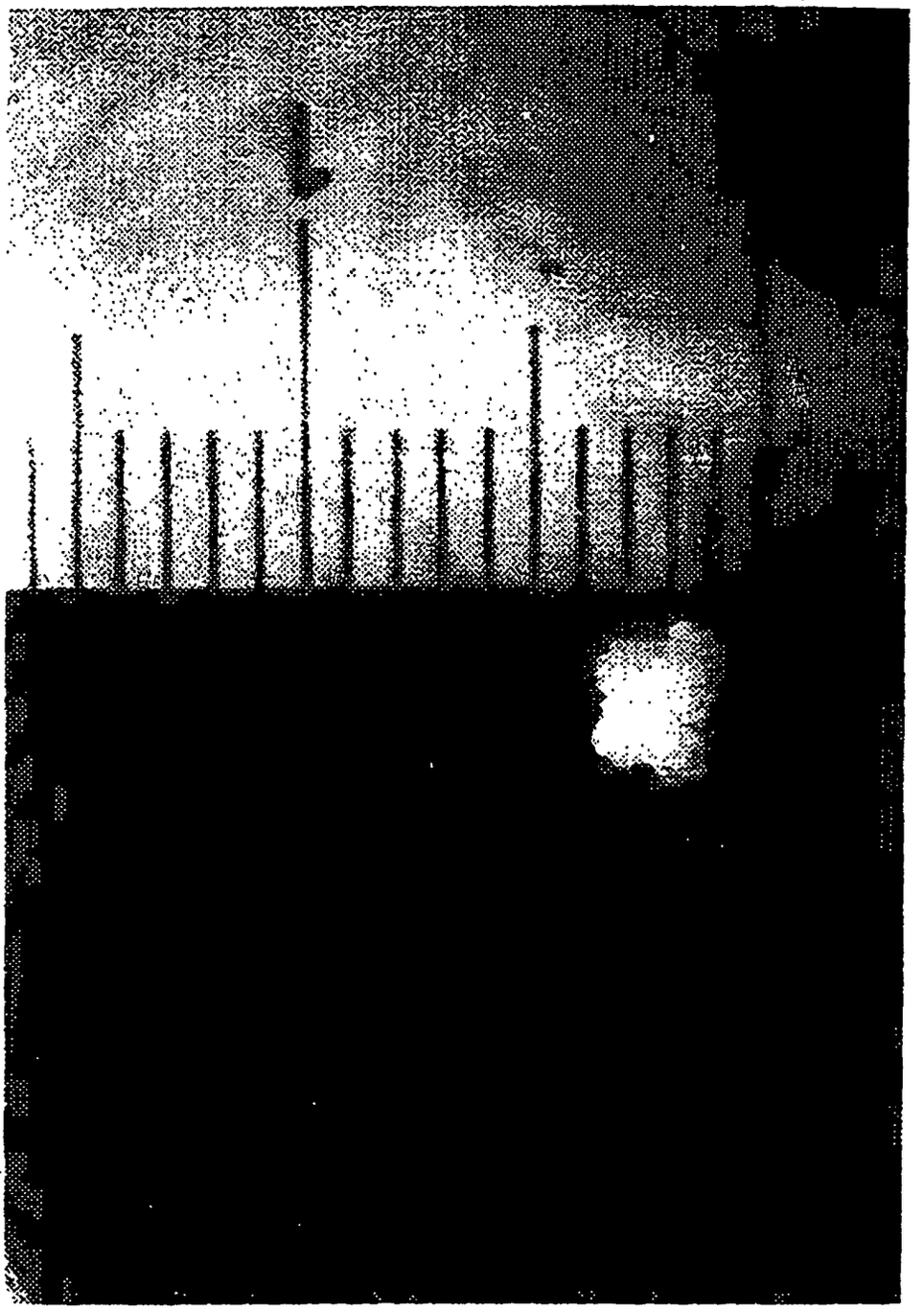


FIG. IA

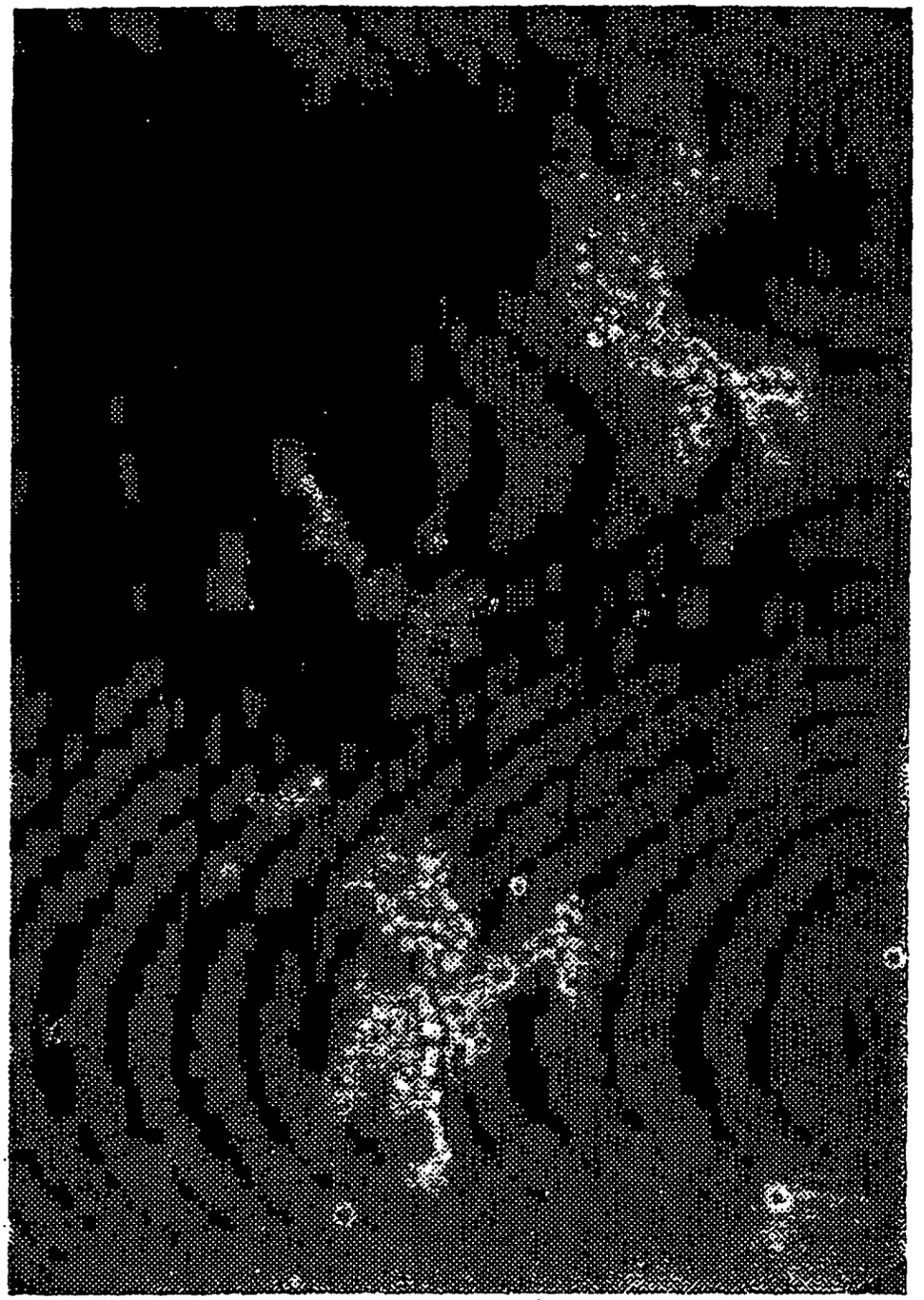


FIG. 1B

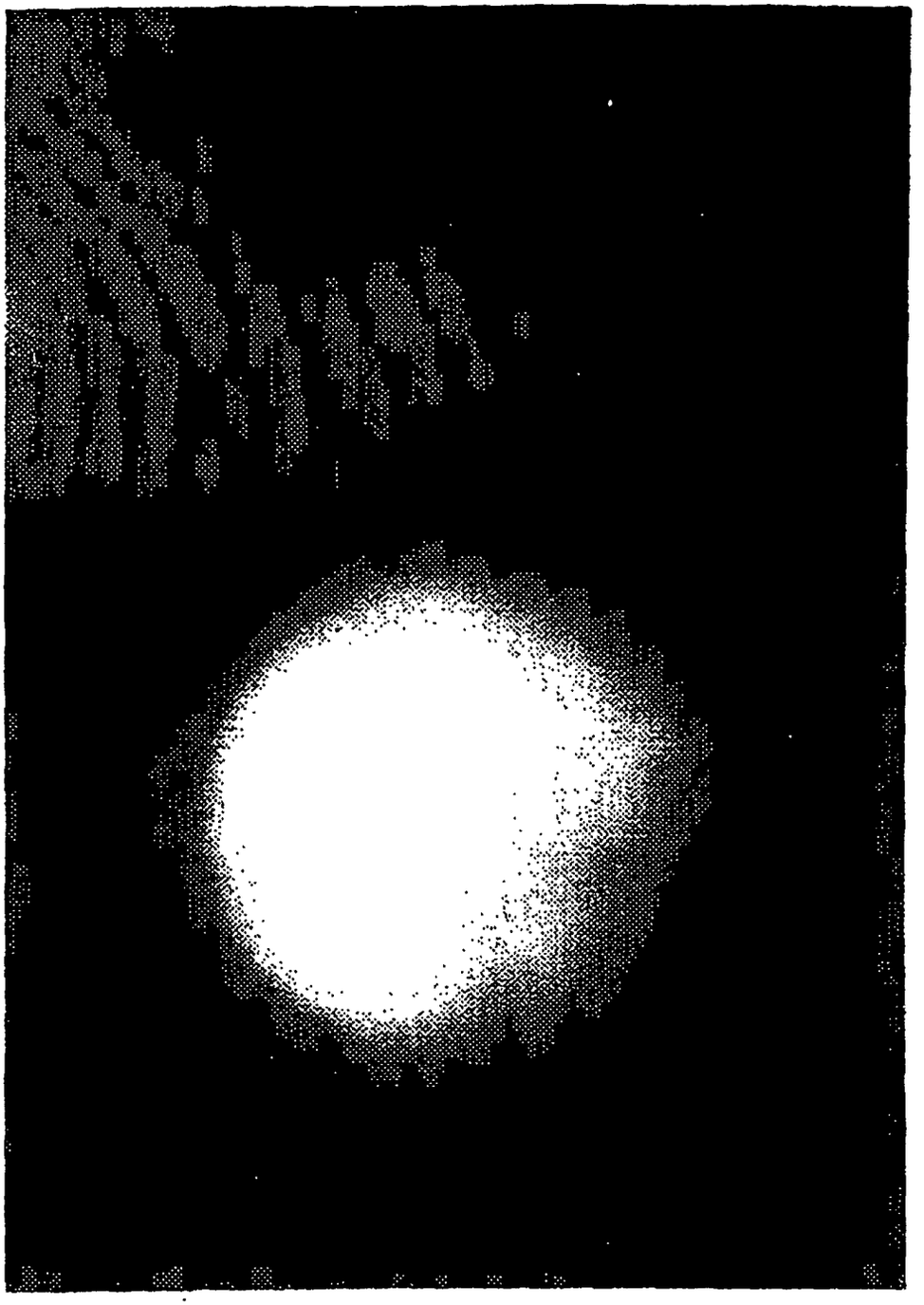


FIG. 2A



FIG. 2B

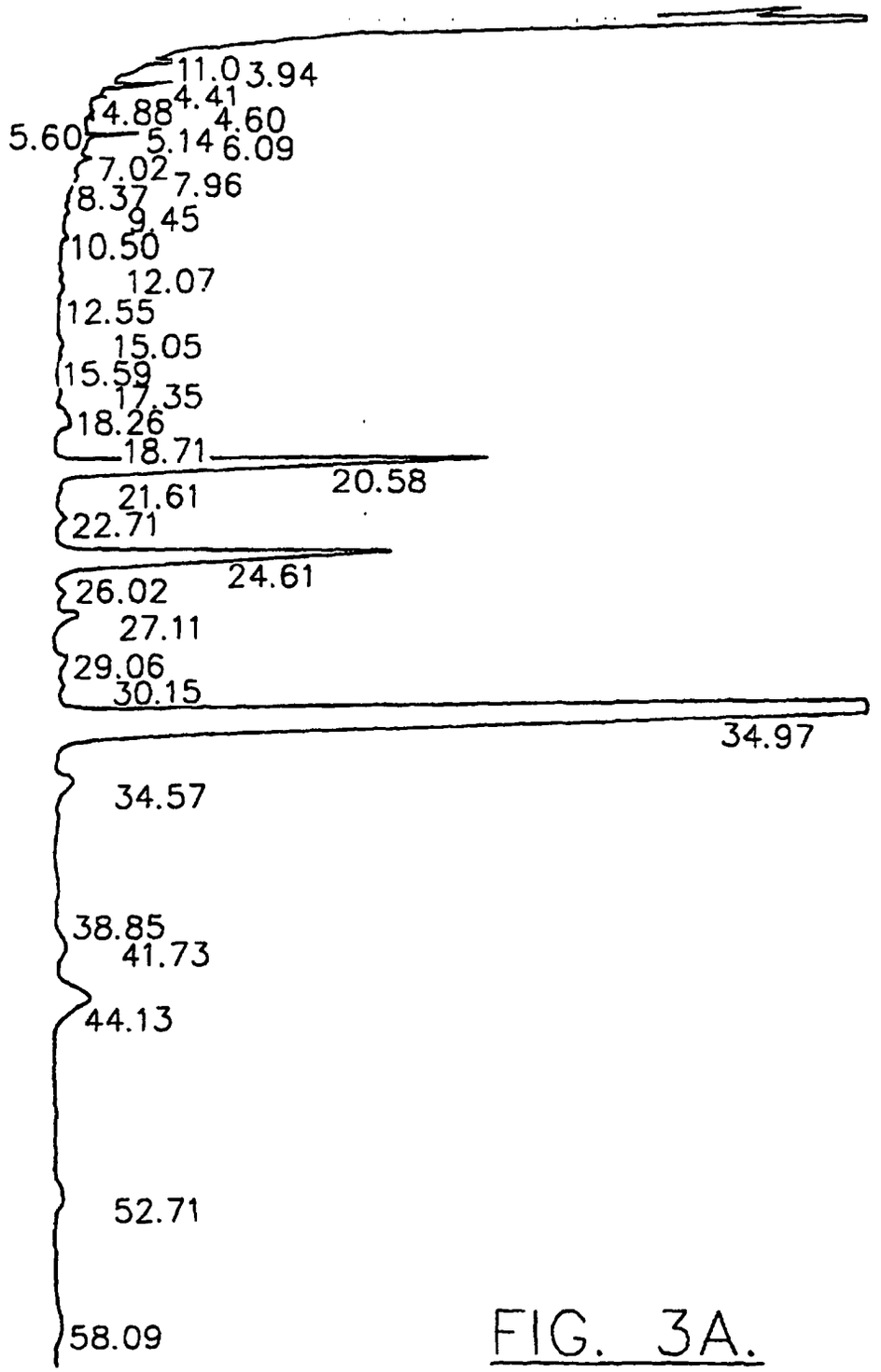

11.0
3.94
4.41
4.88
4.60
5.60
5.14
6.09
7.02
7.96
8.37
9.45
10.50
12.07
12.55
15.05
15.59
17.35
18.26
18.71
20.58
21.61
22.71
24.61
26.02
27.11
29.06
30.15
34.97
34.57
38.85
41.73
44.13
52.71
58.09

FIG. 3A.

SAMPLE 1 CYCS13B1 BIN 9 NAME ARUN0009

SA	IS	XF
27.5	0	74.5

NAME	mcg/ml	RT	AREA	BC	RF	
1	0.712	3.94	4178	01	15899.734	
2	21.286	4.11	124931	03	15899.734	
3	4.056	4.6	23803	01	15899.734	
4	0.65	4.88	3815	02	15899.734	
5	6.553	5.14	38460	03	15899.734	
6	1.637	5.6	9606	01	15899.734	
7	35.318	6.09	207285	01	15899.734	
8	9.726	7.02	57079	01	15899.734	
9	3.173	7.96	18621	02	15899.734	
10	2.533	8.37	14871	03	15899.734	
11	3.738	9.45	21936	01	15899.734	
12	3.764	10.5	22091	01	15899.734	
13	3.979	12.07	23355	02	15899.734	
14	3.625	12.55	21272	03	15899.734	
15	6.379	15.05	37443	02	15899.734	
16	3.611	15.59	21193	03	15899.734	
17	8.554	17.35	50201	02	15899.734	
18	11.735	18.26	68872	02	15899.734	
19	23.898	18.71	140260	03	15899.734	
20	579.379	20.58	3400390	08	15899.734	← CYCLOSPORIN C
21	3.262	21.31	19148	05	15899.734	
22	17.741	22.71	104123	01	15899.734	
23	545.328	24.61	3200541	02	15899.734	← CYCLOSPORIN B
24	17.826	26.02	104626	02	15899.734	
25	59.788	27.11	350898	08	15899.734	
26	25.322	29.06	148619	06	15899.734	
27	12.095	30.15	70987	06	15899.734	
CYCA	3014.026	31.967	17689407	08	15899.734	← CYCLOSPORIN A
29	40.068	34.57	235161	05	15899.734	
30	16.613	38.85	97498	02	15899.734	
31	40.238	41.73	240266	02	15899.734	
32	120.718	44.13	708468	03	15899.734	← CYCLOSPORIN D
33	24.755	52.71	145290	01	15899.734	
34	25.138	58.09	147537	01	15899.734	
TOTALS	4697.919		27572226			

FIG. 3B.

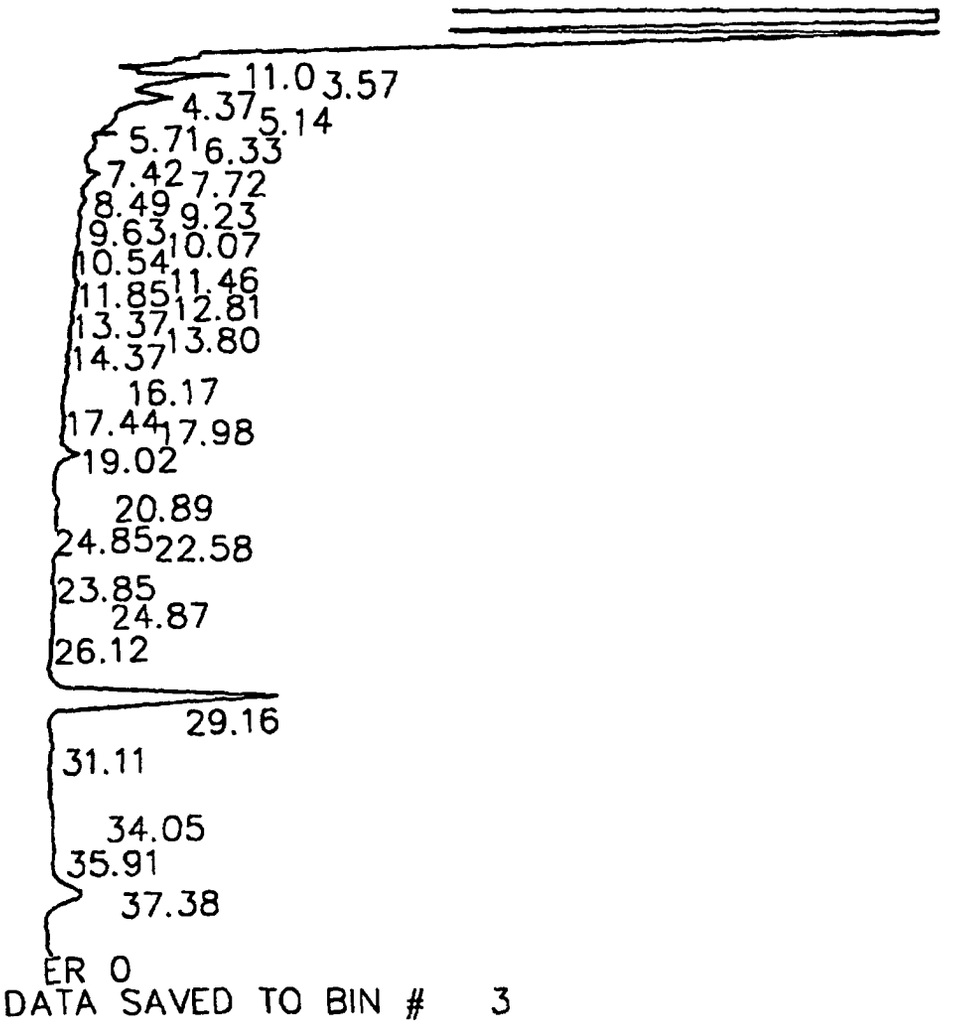

11.0
3.57
4.37
5.14
5.71
6.33
7.42
7.72
8.49
9.23
9.63
10.07
10.54
11.46
11.85
12.81
13.37
13.80
14.37
16.17
17.44
17.98
19.02
20.89
24.85
22.58
23.85
24.87
26.12
29.16
31.11
34.05
35.91
37.38
ER 0
DATA SAVED TO BIN # 3

FIG. 4A.

SAMPLE 1 SNUZ BIN 3 NAME ARUN0003

SA	IS	XF
27.5	0	73.5

1	28.813	3.57	168086	01	15592.097	
2	42.947	4.37	250544	01	15592.097	
3	4.934	5.14	58788	01	15592.097	
4	7.905	5.71	46170	01	15592.097	
5	7.213	6.33	42084	01	15592.097	
6	20.67	7.42	120582	02	15592.097	
7	3.897	7.72	22732	03	15592.097	
8	6.841	8.49	39908	01	15592.097	
9	0.71	9.23	4143	02	15592.097	
10	4.632	9.63	27025	02	15592.097	
11	2.584	10.07	15073	08	15592.097	
12	0.264	10.54	1538	05	15592.097	
13	0.72	11.46	4303	02	15592.097	
14	5.191	11.85	30285	02	15592.097	
15	2.23	12.81	13005	02	15592.097	
16	2.476	13.37	14446	02	15592.097	
17	2.091	13.8	12201	02	15592.097	
18	5.571	14.37	32495	03	15592.097	
19	2.035	16.17	11876	01	15592.097	
20	3.554	17.44	20733	02	15592.097	
21	10.237	17.98	59718	02	15592.097	
22	29.355	19.02	171253	03	15592.097	← CYCLOSPORIN C
23	5.992	20.89	34954	02	15592.097	
24	6.892	21.85	40207	02	15592.097	
25	33.671	22.58	196427	03	15592.097	← CYCLOSPORIN B
26	0.971	23.85	5666	02	15592.097	
27	7.936	24.87	46297	02	15592.097	
28	8.634	26.12	50372	03	15592.097	
CYCA	385.065	29.16	2246382	02	15592.097	
30	9.045	31.11	52767	02	15592.097	
31	5.693	34.05	33208	02	15592.097	← CYCLOSPORIN A
32	21.739	35.91	126821	02	15592.097	
33	78.59	37.38	458476	03	15592.097	← CYCLOSPORIN D
TOTALS	759.108		4428465			

FIG. 4B.